Chlorpyrifos effects at different levels of ecological hierarchy: link between changes in biomarkers (sub-organism level) and behavioral changes in *Daphnia magna* (organism level)

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Aims of the project

Emerging Contaminants (ECs) sub-lethal concentrations

Evidences that the exposure to 'environmentally relevant' concentrations of different ECs can induce several adverse effects to non-target organisms[1].

Can sub-lethal concentrations of ECs induce biomarker changes in non-target organisms ? (sub-organism level)

Can sub-lethal concentrations of ECs induce behavioral changes in non-target organisms? (organism level)

Are these potential changes linked?

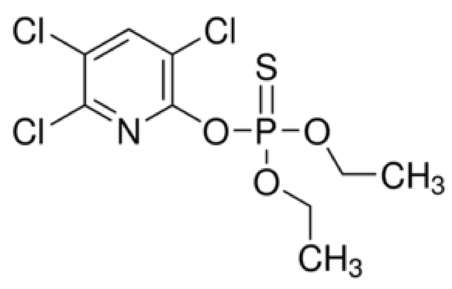
Biological target

Daphnia magna

Descripiton: small planktonic crustacean (adult length 1.5–5 mm) which reproduces by parthenogenesis.
Laboratory breeding: 40 individuals/L in a commercial mineral water (San Benedetto®) fed *ad libitum* with a suspension of the unicellular green algae *Pseudokirchneriella subcapitata* and the yeast *Saccharomyces cerevisiae* three times a week.

Contaminant

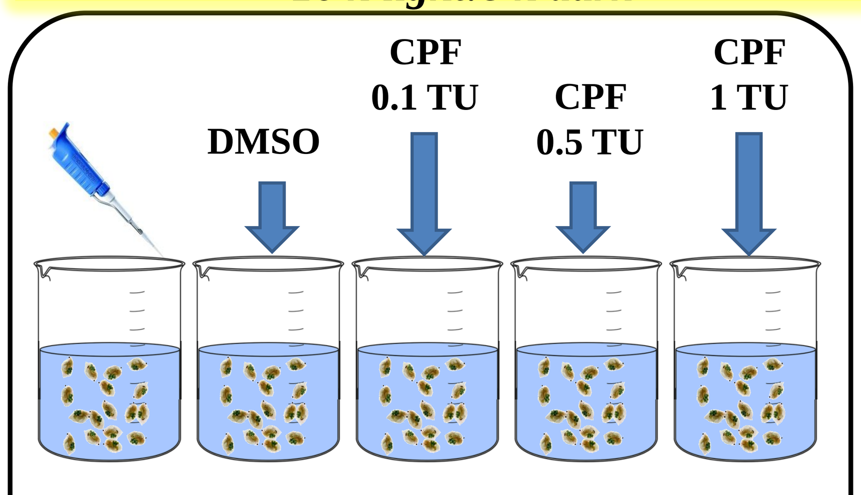
Chlorpyrifos (CPF)



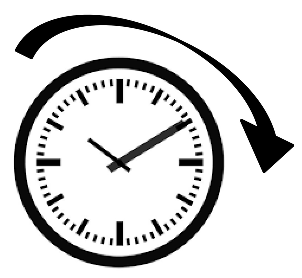
Organophosphate pesticide: insecticide, acaricide, miticide
Mechanism of action: acetylcholinesterase inhibition
Major crops: cotton, corn, fruit trees
Environmental impact: POP candidate for its persistence, bioaccumulation, toxicity and potential for atmospheric long-range transport [2].

CPF-exposure

16 h light:8 h dark *T: 20 ± 2 °C*

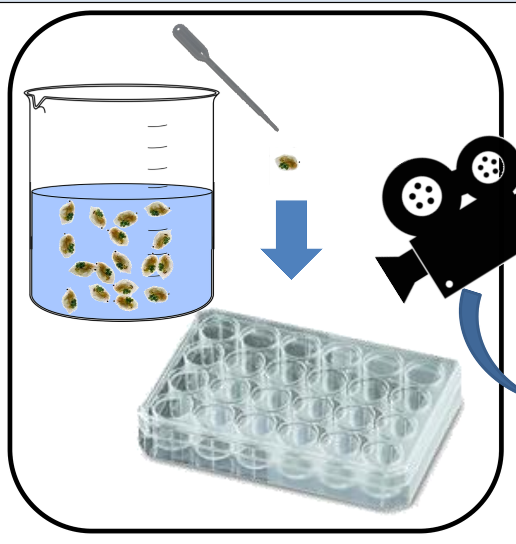


CPF concentrations assessed: 0.1 TU (50 ng/L), 0.5 TU (250 ng/L) 1 TU (500 ng/L)

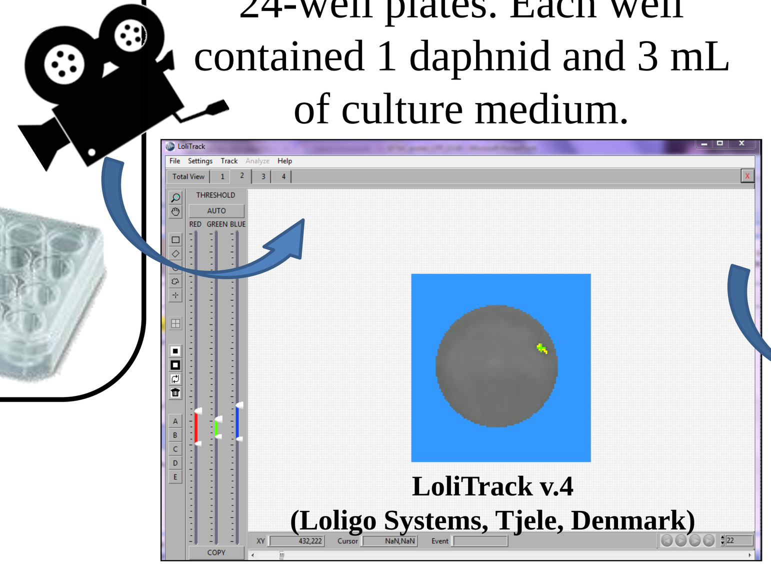


96 hours

Video-tracking analysis



At the end of the exposure, organisms were transferred into 24-well plates. Each well contained 1 daphnid and 3 mL of culture medium.



Biomarkers analysis

Biomarker analysis was performed on homogenates from a pool of all the alive daphnids found in each beaker .
We analyzed the activity of:

- antioxidant enzymes (SOD, CAT and GPx)
- detoxifying enzyme (GST)
- acetylcholinesterase (AChE)

according to spectrophotometric methods.

Results

Biomarker changes

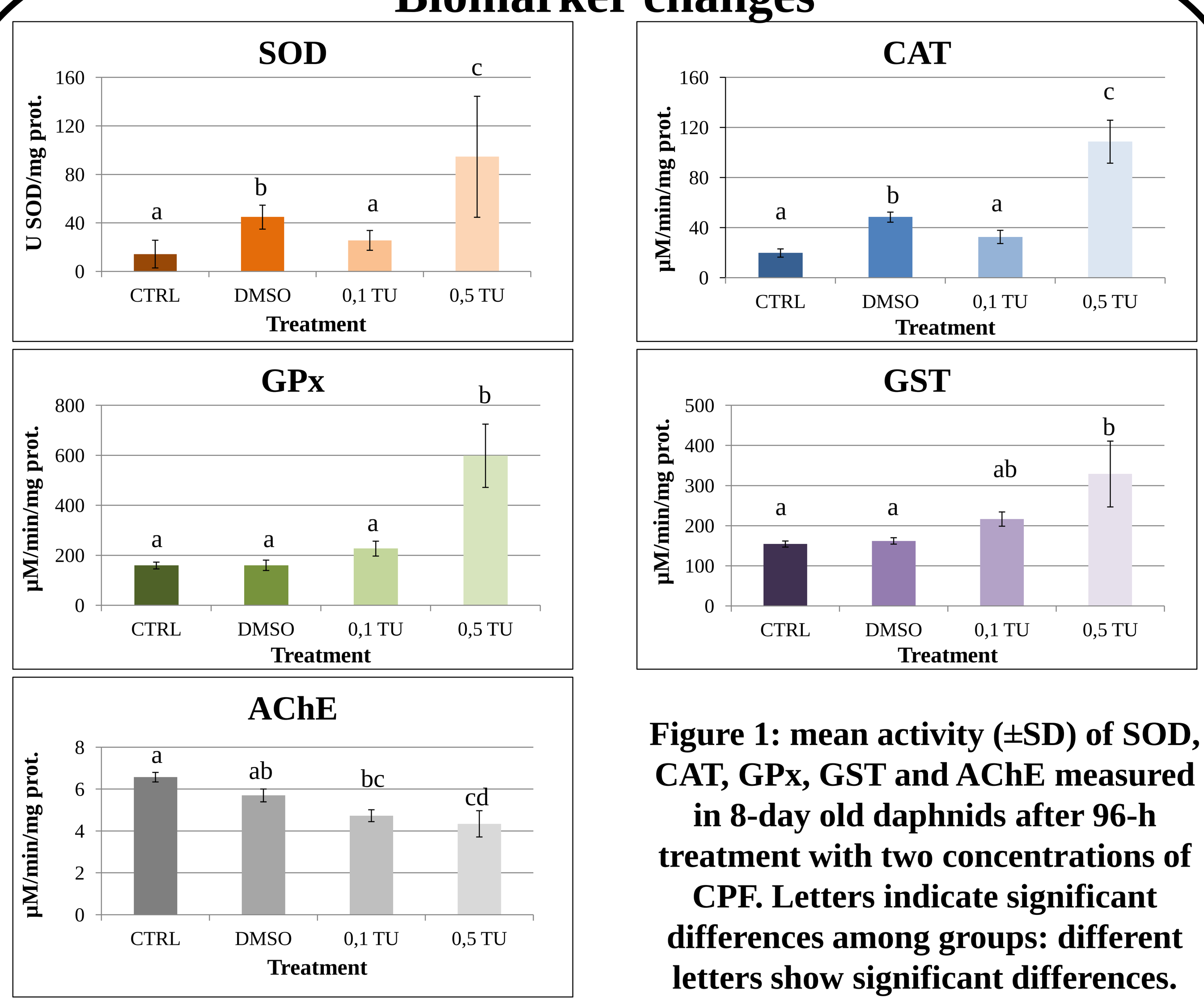


Figure 1: mean activity (±SD) of SOD, CAT, GPx, GST and AChE measured in 8-day old daphnids after 96-h treatment with two concentrations of CPF. Letters indicate significant differences among groups: different letters show significant differences.

Viable daphnids accounted on average for more than 90% of the population at the beginning of the experiment, with exception of the exposure to 1 TU of CPF. Under this exposure condition, only three daphnids out of forty we exposed have survived and then the amount of proteins available was not enough for biomarker analysis.

Biomarker changes (sub-organism level)

A significant effect of treatment was found for SOD ($F = 9.723$; $p < 0.01$), CAT ($F = 58.310$; $p < 0.01$), GPx ($F = 35.041$; $p < 0.01$), GST ($F = 9.113$; $p < 0.01$) and AChE activity ($F = 6.483$; $p < 0.05$).

- **Antioxidant and detoxifying enzymes:**
  - 0.1 TU of CPF did not cause a significant modulation of antioxidant and detoxifying enzymes ($p > 0.05$ in all the cases, with exception of a decrease of CAT compared to DMSO);
  - 0.5 TU of CPF induced a 2- to 4-fold significant increase of SOD, CAT, GPx and GST with respect to both negative control and DMSO.
- **Acetylcholinesterase inhibition:**
  - 0.1 TU of CPF had not a significant effect on AChE activity of daphnids compared to DMSO;
  - 0.5 TU of CPF showed a significant inhibition of about -24% respect to negative control at the end of the exposure.

Behavioral changes

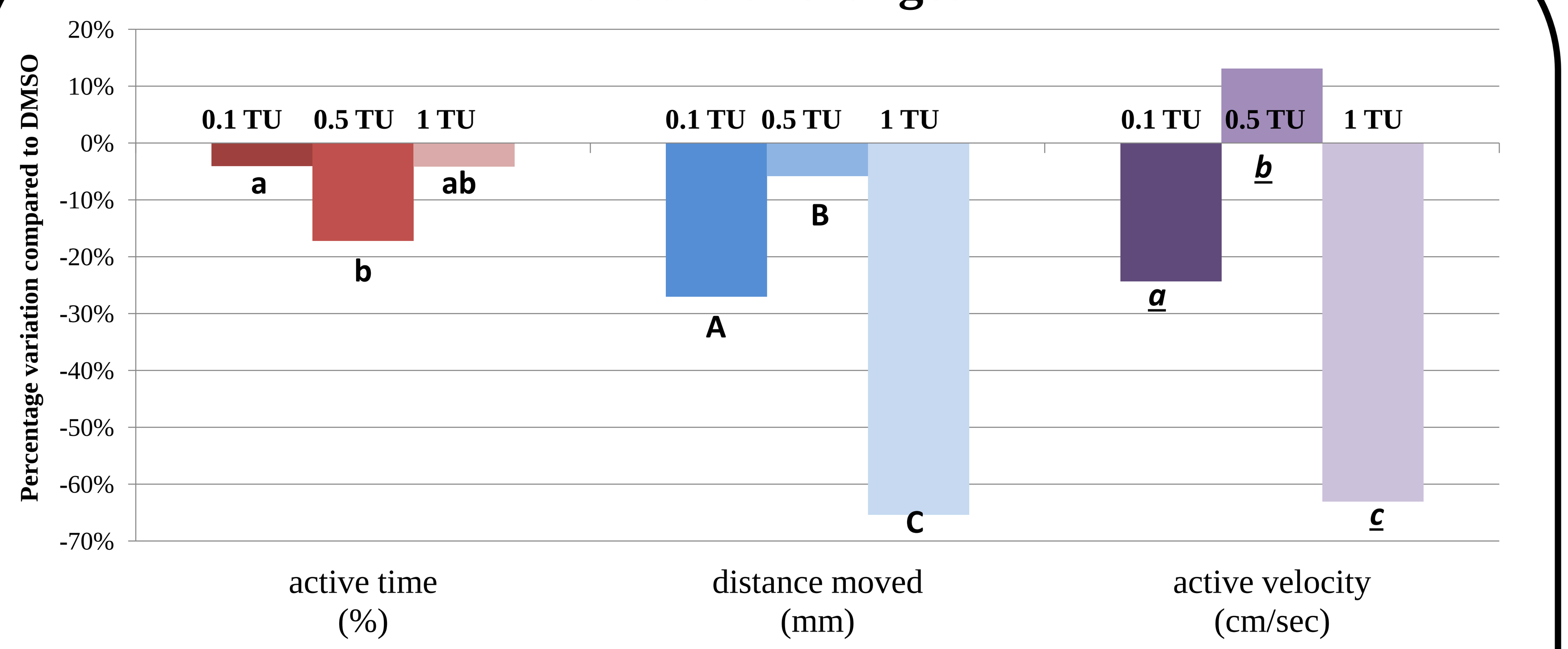


Figure 2: mean active time (%), distance moved (mm) and velocity (cm/sec) measured in 8-day old daphnids after 96-h treatment . Results are showed as the percentage of variation of each single endpoint measured in treated individuals compared to DMSO. Letters indicate significant differences among groups: different letters show significant differences.

Behavioral changes (organism level)

CPF had a significant effect on daphnids' behavior compared to DMSO.

- **Active time:** CPF induced a decline of daphnids active time, that decreased significantly at 0.5 TU of CPF. 1 TU of CPF caused a general decline of active time with exception of one daphnid showing an hyperstimulation;
- **Distance moved:** 0.5 TU of CPF had not a significant effect of distance traveled by daphnids contrary to the other assessed concentrations.
- **Active velocity:** the active velocity is calculated from positive velocity values only and then represents the ratio between active time and distance moved. This parameter confirmed that all evaluated concentrations of CPF had a significant effects on daphnids behavior compared to DMSO.

Conclusion

Our findings suggest that environmental concentrations of CPF induced adverse effects on daphnids both at sub-organism and organism level. Based on these results, more studies should be useful to confirm that sub-lethal effects at sub-organism level can lead to behavioral changes in the organism and serve as early warning signal to foresee effects at higher levels of the ecological hierarchy.

References
[1] Parolini M. et al. (2013) Sub-lethal effects caused by the cocaine metabolite benzoylecgonine to the freshwater mussel *Dreissena polymorpha*. Sci. Tot. Environ. ,444, pp. 43–50
[2] Mackay D.et al. (2014) Fate in the Environment and Long-Range Atmospheric Transport of the Organophosphorus Insecticide, Chlorpyrifos and Its Oxon. Rev. Environ. Contam. Toxicol., 231, pp. 35–76